Latest development of Thin Layer Chromatography *at Merck*

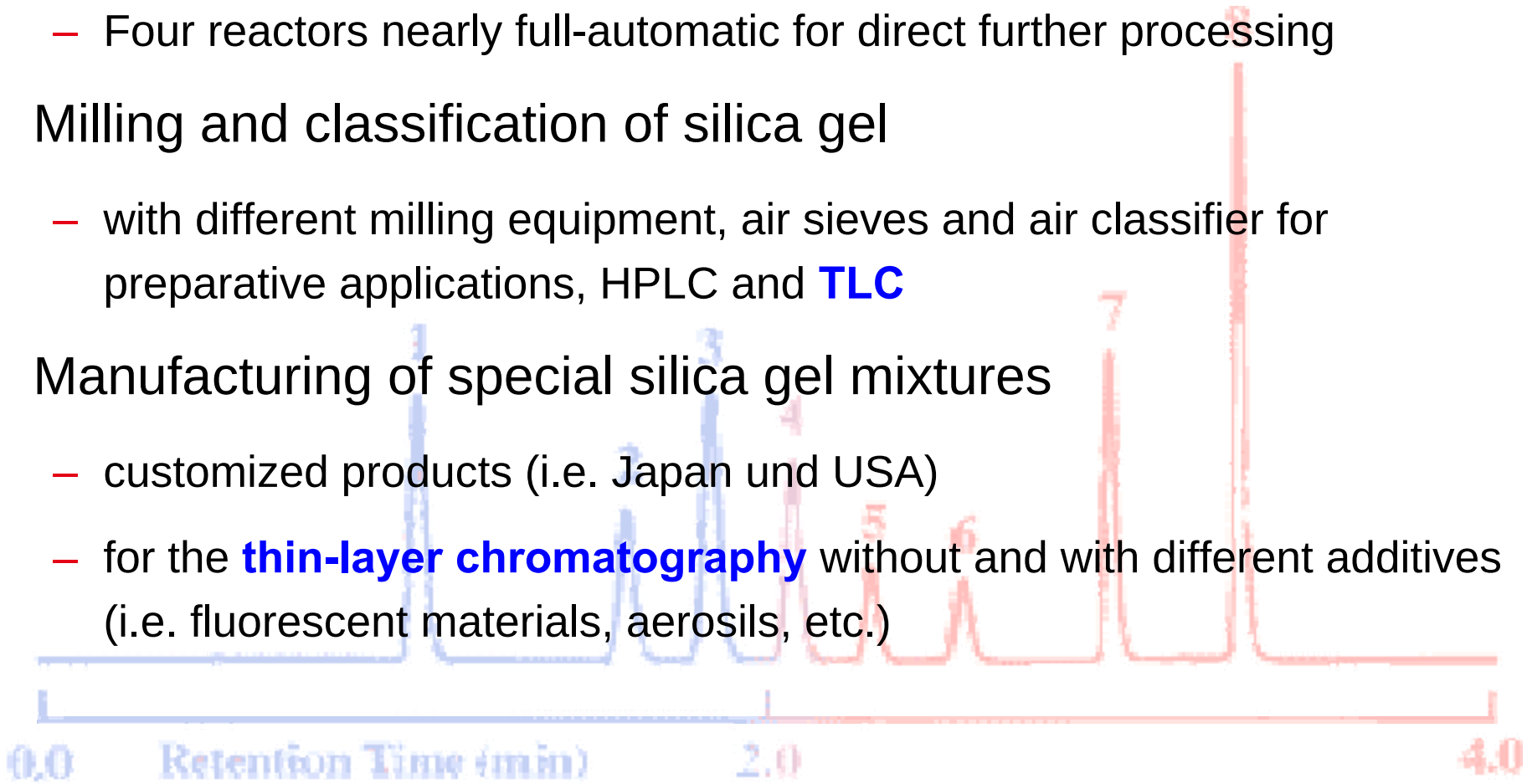
Dr. Mehmet Dogan, PLS/LB- LC

Customer Seminar 2009

Silica and Aluminium oxide production facilities, Gernsheim

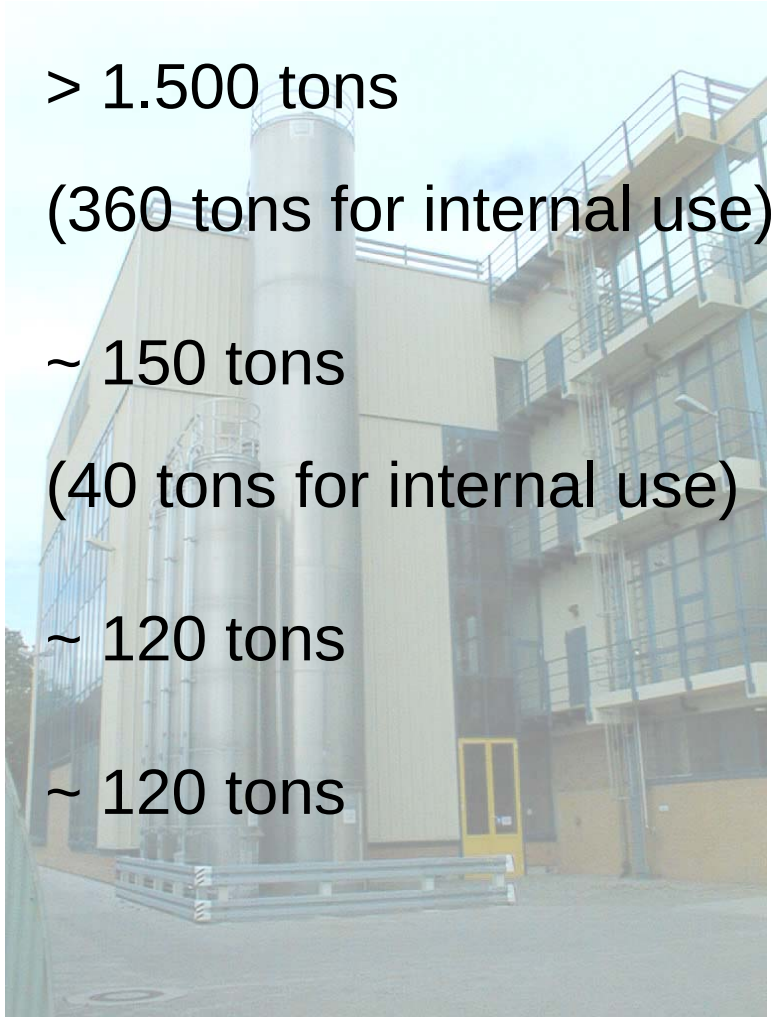
Silica gel Production

- Production of raw silica gel for the preparative chromatography
  - Four reactors nearly full-automatic for direct further processing
- Milling and classification of silica gel
  - with different milling equipment, air sieves and air classifier for preparative applications, HPLC and **TLC**
- Manufacturing of special silica gel mixtures
  - customized products (i.e. Japan und USA)
  - for the **thin-layer chromatography** without and with different additives (i.e. fluorescent materials, aerosils, etc.)



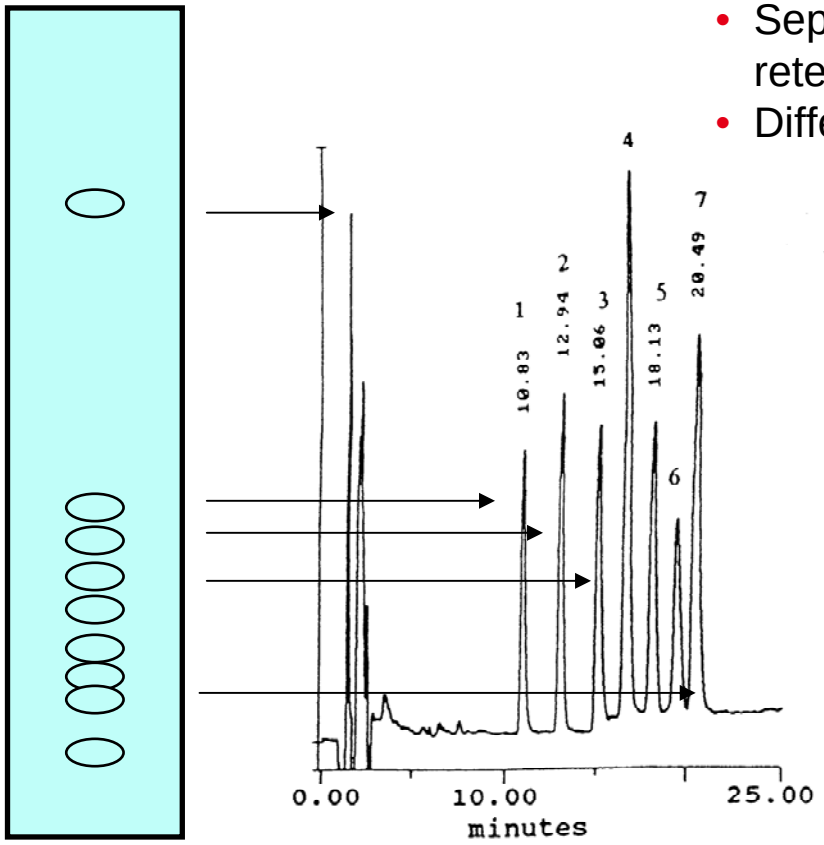
Current output
(with increasing volume)

- Silica gel : > 1.500 tons
  (360 tons for internal use)
- Aluminium oxide: ~ 150 tons
  (40 tons for internal use)
- Products of spray drying : ~ 120 tons
- Miscellaneous products: ~ 120 tons



Method tranfer from TLC to HPLC

Fig. 1: TLC separation (left) and the corresponding HPLC separation (right)



HPLC and TLC

- Separations occur by the same retention mechanism
- Differences arise from:
  - Kinetic performance
  - Stationary phase format
  - Development mode vs. elution
    Disposable stationary phase (TLC)
  - Detection in the presence of the stationary phase (TLC)

Merck
Pioneered Thin Layer Chromatography

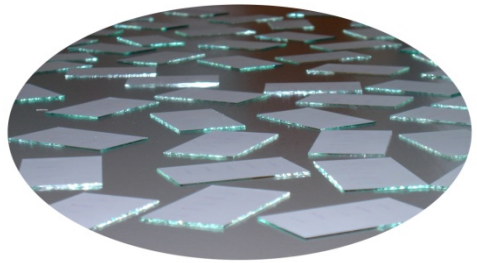
1938	Al_2O_3 layers (Izmailov and Shraiber)
1951	Silica gel layers with calcium sulphate (Kirchner)
1950	Egon Stahl is founder of thin layer Chrom. and standardized silica gels (Higher sensitivity more and universal scope of applications)
1958	Merck launched TLC during Achema exhibition
1966	Pre-coated TLC plates
1975	Pre-coated HPTLC plates
1978	Modified sorbents for TLC and HPTLC
1995	Spherical sorbents for HPTLC (LiChrospher®)
2002	Ultra thin monolithic silica plates (UTLC)
2003	LuxPlate®
2006	ProteoChrom® Plates

First presentation of pre-coated plates, Achema 1958

TLC Production Today

- 20 employes in production plant
- > 7 million plates per year
- Every single plate is visually inspected
- More than 60 different products

On these plates 45 million analyses are carried out each year!



Production Process of TLC Plates

Preparation of suspension of silica gel in water
(eventually with fluorescence indicator)

↓

Coating of plates or sheets
(glass, aluminum, plastic)

↓

Drying in drying tunnel

↓

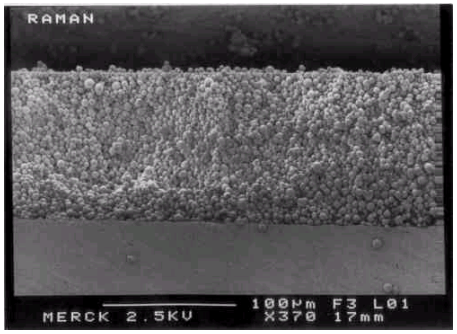
Sampling, in-process control

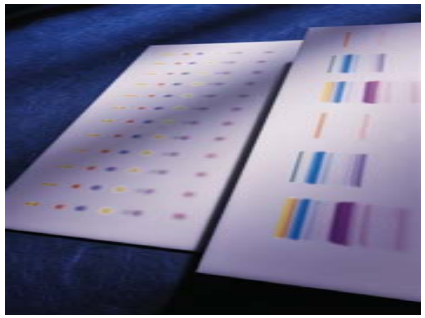
↓

Cutting (for formats smaller than 20 x 20 cm),

↓

Packaging, final control





… a success story …
Thin-layer chromatography

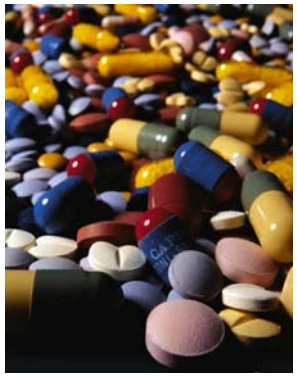
In focus: User-friendliness

- 1966: Merck launches precoated plates for TLC

Precoating TLC plates, 1967

TLC with concentrating zones, 1970s

TLC - Many Application Fields



Pharma & Herbal Medicine

- **R&D / Synthesis Labs**
  Stability testing
  Uniformity testing
  Sub-component evaluation
- **Quality Control / Analytical Labs**
  In-process control
  Identity testing

Environmental Analysis

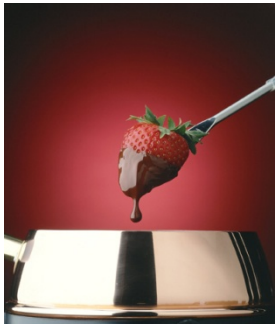
Water & soil analysis
Residue analysis

Clinical Labs

Drug monitoring
Metabolism studies
Doping control

Forensic

Drug of Abuse,
Poisons, Alkaloids



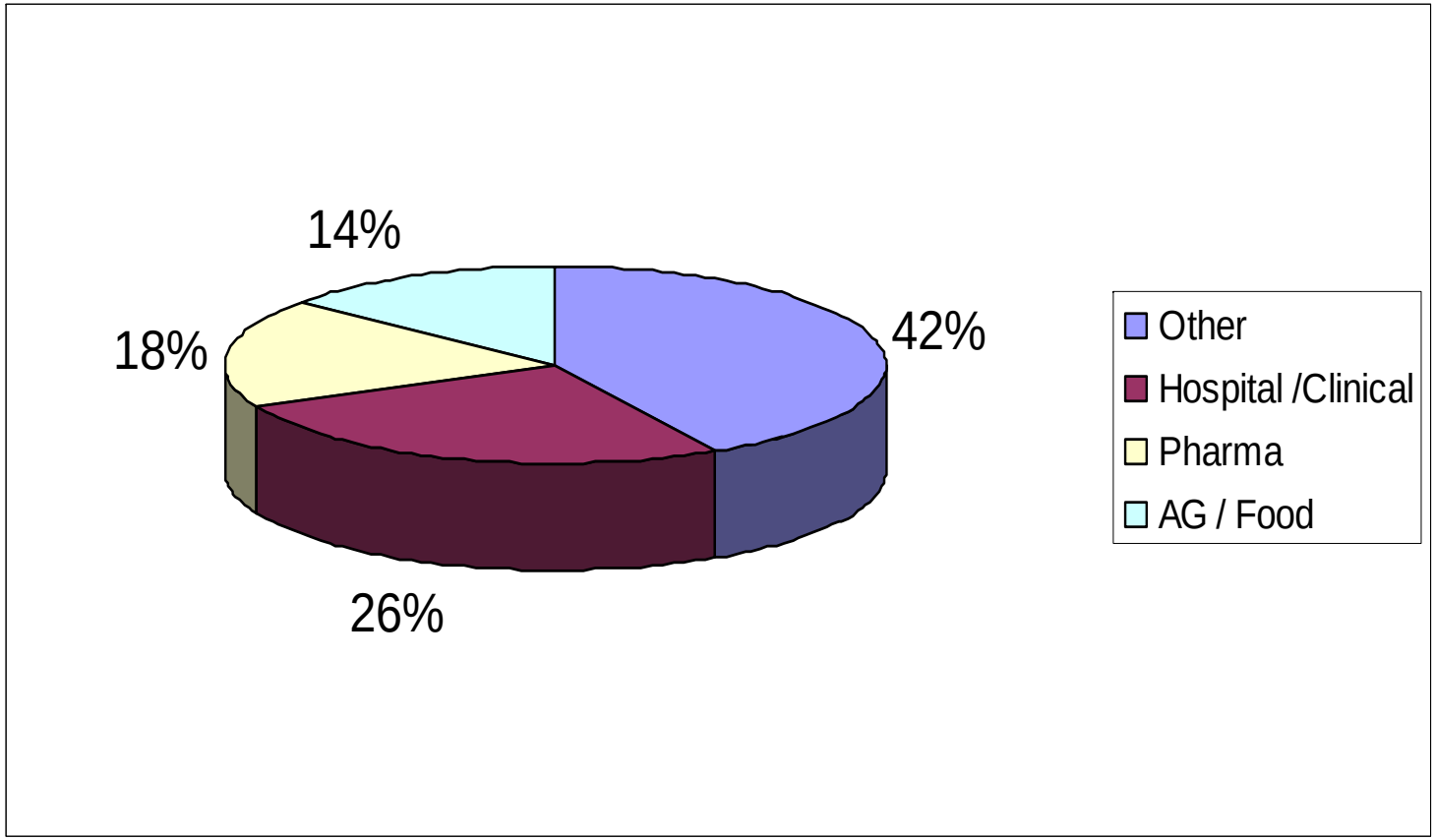
Food

- **Quality control**

Stability testing
Drug residue testing
Testing for additives
Mycotoxins (including aflatoxins)

Market Thin Layer Chromatography

Total 40 – 50 Mio EUR
AGR: 2 %



SDi Global Assessment Report 9th Edition, LCGC Oct.08

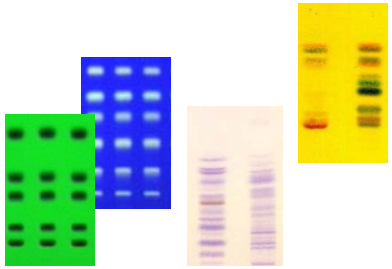
Thin Layer Chromatography

- Stationary phase is a thin layer of sorbent coated on an inert backing material
- Sample is applied to the layer as a spot or band near to the bottom edge
- Separation occurs in an enclosed chamber by contacting the bottom edge of the layer by the mobile phase
- Separation results from the different rates of migration of the sample components in the direction traveled by the mobile phase
- Sample components are identified based on their position in space

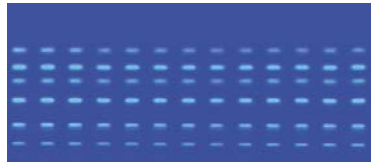
TLC – as the First Choise

- Fast separations - no need for sophisticated instruments

- Direct visualisation of results by either UV or staining (Postchromatographic reaction)

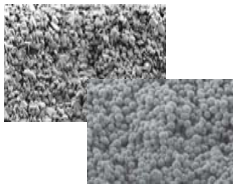


- Simultaneous analysis of many samples in parallel under the same conditions



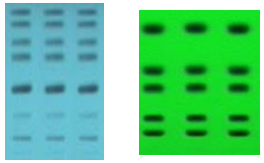
- No need sample preparation step because TLC plates are disposables

TLC Range at a Glance

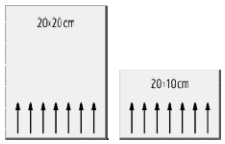


- Sorbens types
  - **Silica 60**
  - Modified silica: RP2, RP8, **RP18,** NH_2, Diol, CN;
  - Aluminium oxide, Cellulose

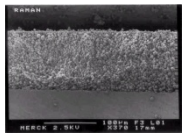
- Backing (support)
  - Glass
  - Aluminium (plastic)



- Detection
  - with fluorescence indicator F_{254}:green, F_{254s}: blue, (F_{366}:blue)



- Plate sizes (in cm)
  - 20 x 20, 10 x 20, 5 x 10 ….. 5 x 7,5, 2,5 x 7,5



- Plate thickness
  - TLC: 250 µm, **HPTLC:** 200 µm, 100 µm, **UTLC:** 10 µm, **PLC:** 0,5 – 2 mm

TLC Technologies

The separation efficiency of a TLC plate can be improved by:

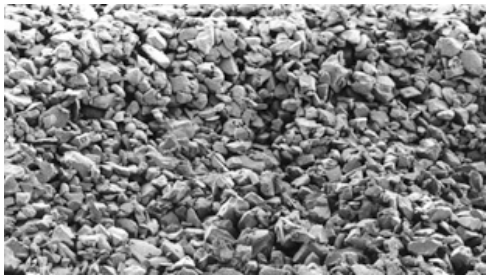
- Mean particle size of the silica sorbent
- Particle size distribution
- Layer thickness

TLC: HPTLC: UTLC:	Classical thin layer chromatography High performance thin layer chromatography Ultra-thin layer chromatography	Analytical
PLC:	Preparative layer chromatography	Preparative

TLC Quality Grades
Silica gel 60 types

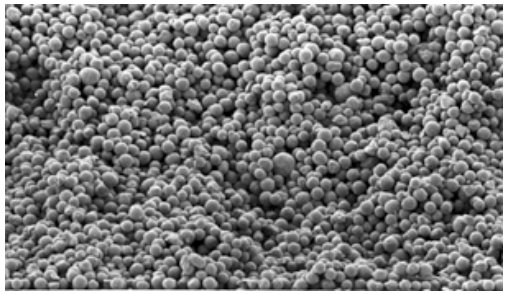
Particle size distribution:

- Classical TLC
  5 - 20 µm

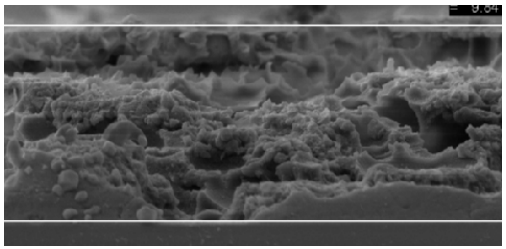


- HPTLC
  4 - 8 µm

- Spherical particles HPTLC
  4 - 8 µm



- Monolithic layer UTLC



Quality

Not comparable

Sorbens Types

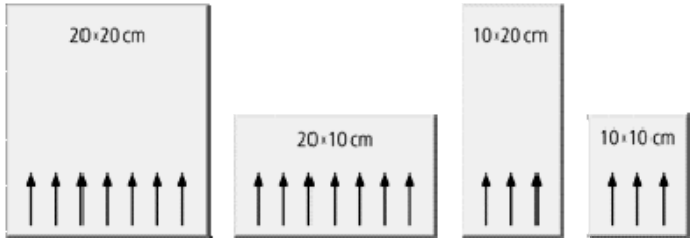
TLC	HPTLC	PLC
Silica gel 60	Silica gel 60	Silica gel 60
$Al2O_3$ 60/150	Al_2O_3 60/150	
Cellulose	Cellulose	
(Kieselguhr)		
RP-2	RP-2	
RP-8	RP-8	
RP-18	RP-18	RP18
	RP-18W	
NH_2	NH_2	
	CN	
	DIOL	

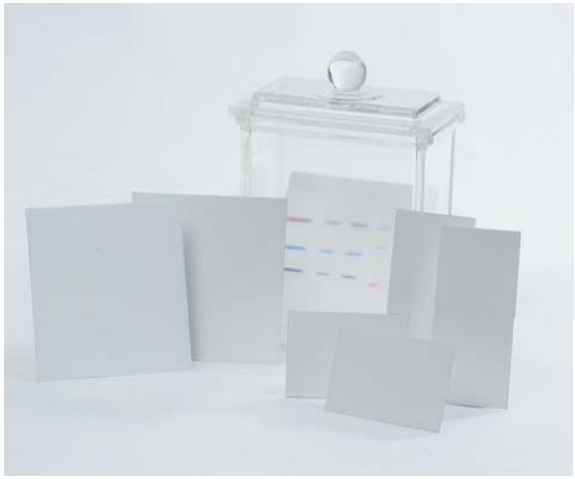
Backings
Glass, aluminium or plastic?

Support	Advantage
Glass	• no bending best for instrumental HPTLC • inert material • temperature stable
Aluminium Plastic	• 20% lower priced then glass • simple to cut with scissors allowing for different formats

Plate Sizes Fitting the Application

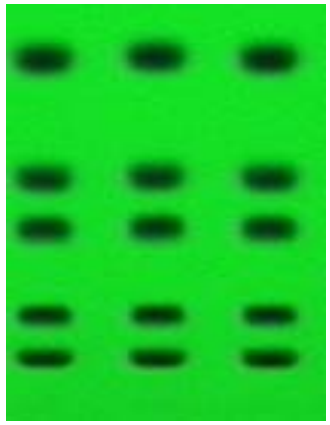
Backing	classical TLC	HPTLC	PLC
Glass	20 x 20 cm 10 x 20 cm 5 x 20 cm 5 x 10 cm 2,5 x 7,5 cm	20 x 10 cm 10 x 10 cm 5 x 10 cm 5 x 5 cm	20 x 20
Aluminium	20 x 20 cm 10 x 20 cm 5 x 20 cm 5 x 10 cm 5 x 7,5 cm	20 x 20 cm 5 x 7,5 cm	
Plastic	20 x 20 cm 500 x 20 cm 4 x 8 cm		



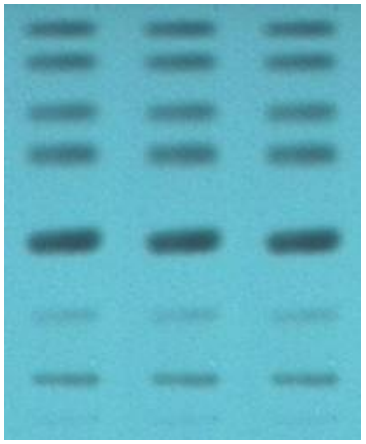


Detection
By UV of colourless substances

- Green fluorescent indicator F_{254}



- Blue fluorescent indicator F_{254s}

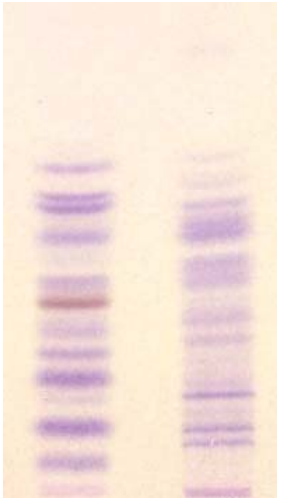


Sample that adsorb UV light are detected due to fluorescence quenching under the UV lamp

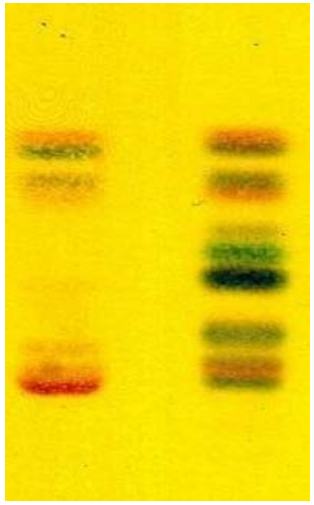
Detection
By Derivatisation / Staining

Many staining options

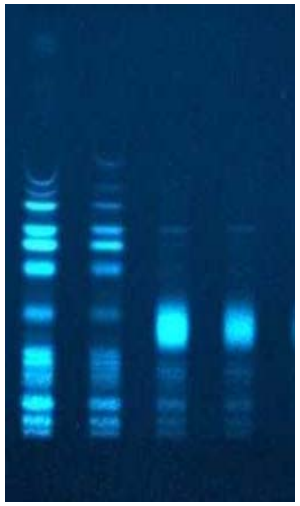
Ninhydrin



Isatin



Fluram



Classical TLC or HPTLC ?

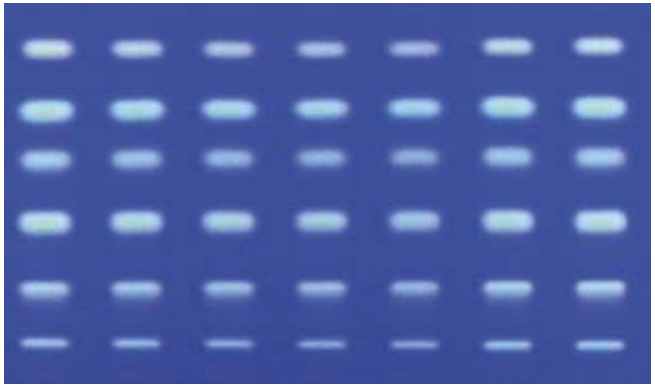
	Classical TLC	HPTLC (High performance TLC)
Application	Quick, inexpensive, flexible and portable separations	Highly sophisticated separation problems, complex samples
Analysis	Qualitative analysis	Qualitative & quantitative analysis
Detection	Visual analysis with UV lamp. Virtually no Instrumentation required	Instrumented analysis: use of scanners for detection
Price	Lower priced (25%)	Higher priced

Instruments suppliers: CAMAG, (DESAGA)

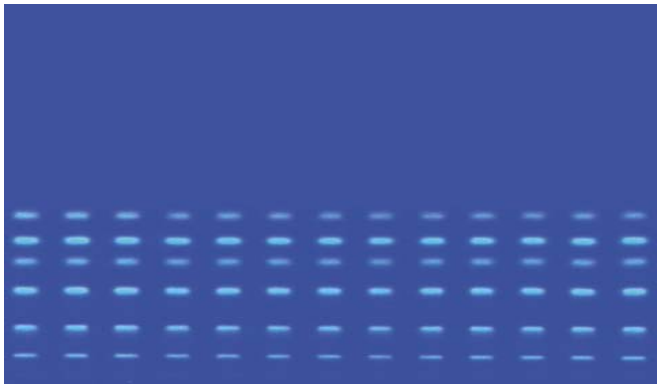
HPTLC versus TLC

- 5 – 10 fold increased sensitivity than classical TLC
- Faster analysis (only 15 min compared to 45 min)
- Gold standard for automated use with instrument

Classical TLC silica gel 60 plate

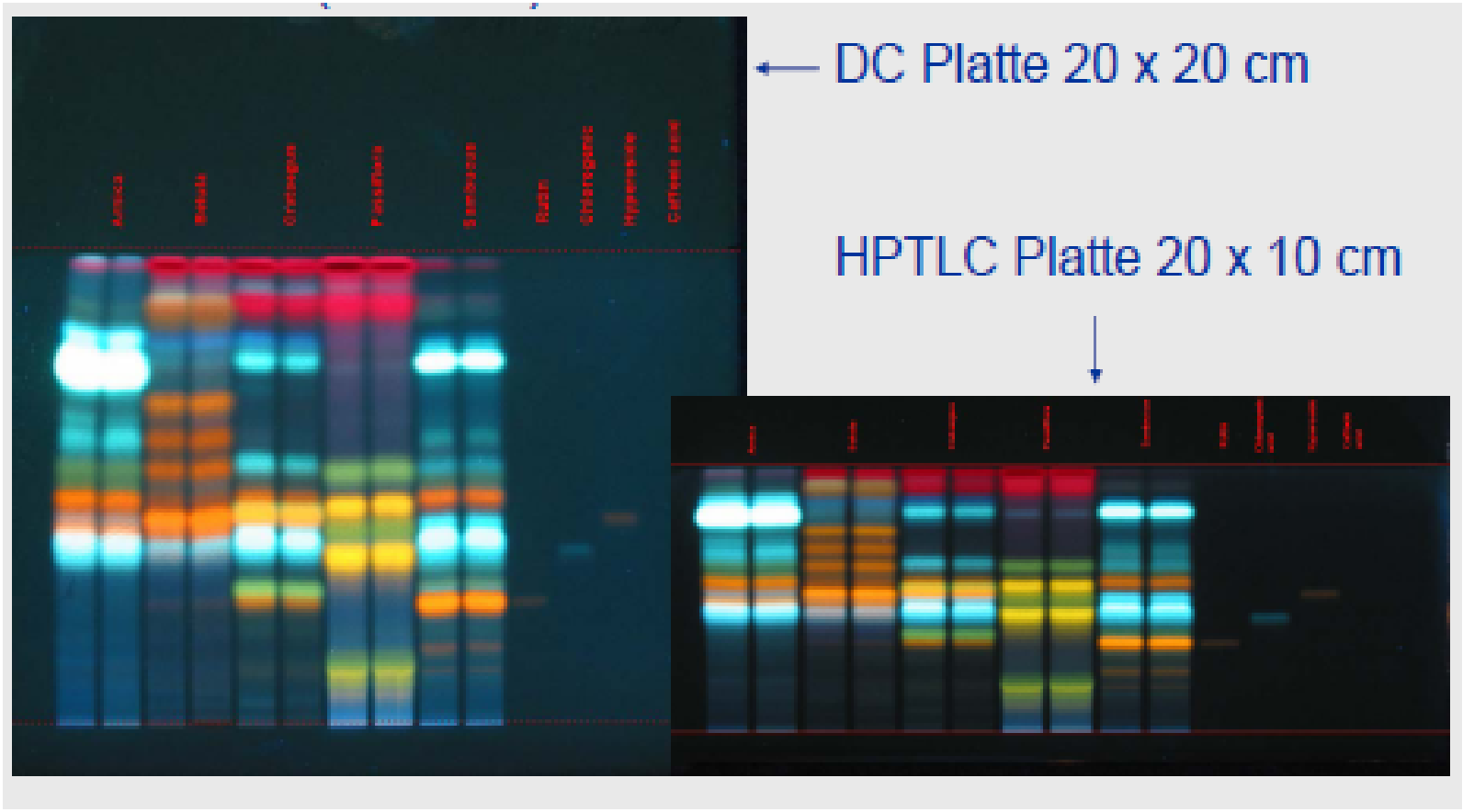


HPTLC silica gel 60 plate



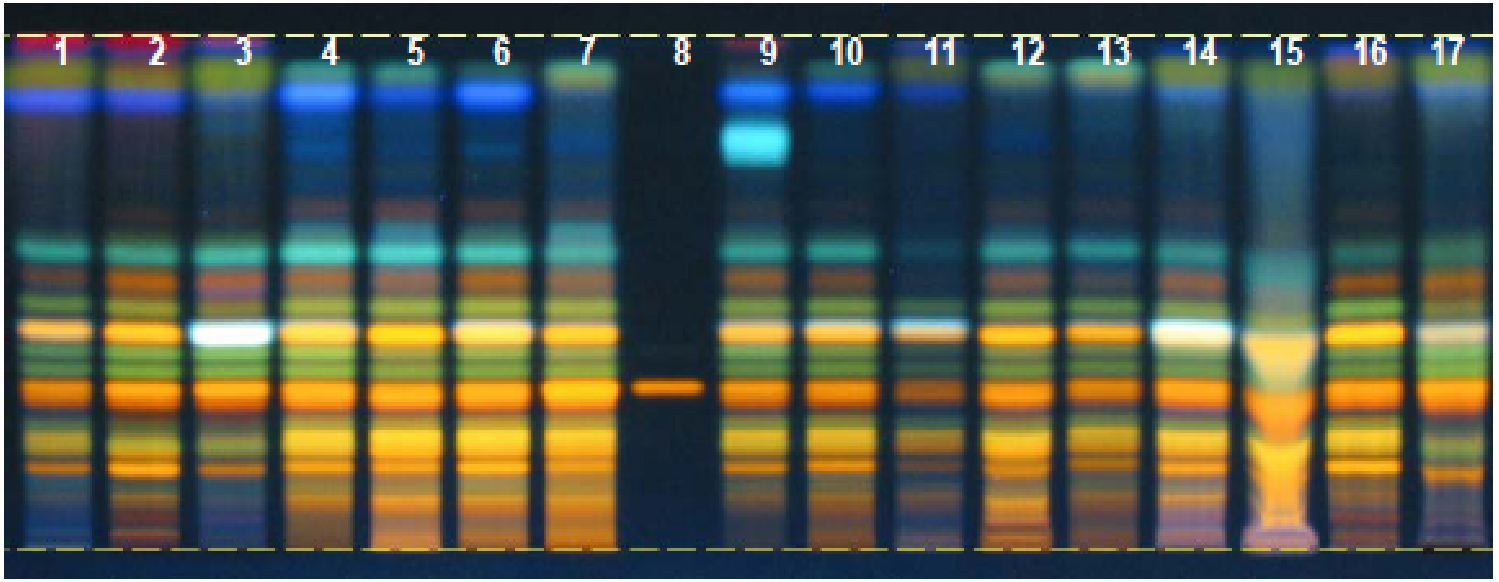
Sample: Separation of dansyl amino acids

Comparison TLC / HPTLC



HPTLC Applications – Herbals

Example: Identification of Gingko



c) UV 366nm, after derivatization with natural products reagent/PEG

1, 2: Ginkgo leaf, 3: Ginkgo leaf capsule (freeze dried; 1.2-1.8% flavonoids; US), 4: Ginkgo leaf extract powder (Italy), 5: Ginkgo leaf extract powder (China), 6: Ginkgo leaf extract powder (France), 7: Ginkgo leaf extract powder (China), 8: Rutin, 9: Ginkgo leaf extract capsule (60 mg) w/gotu kola (US), 10: Ginkgo leaf extract capsule (60 mg; US), 11: Ginkgo leaf extract tablet (yielding 9 mg flavone glycosides; Switzerland), 12: Ginkgo leaf extract tablet (120 mg; US), 13: Ginkgo leaf extract tablet (120 mg; US), 14: Ginkgo tincture (1:5 dry leaf; US), 15: Ginkgo tincture (1:1 fresh leaf; US), 16: Ginkgo tincture (1:10 fresh leaf, Switzerland: current batch), 17: Ginkgo tincture (1:10 fresh leaf, Switzerland: 2 years past expiration date)

Modern Thin layer Chrom. HPTLC

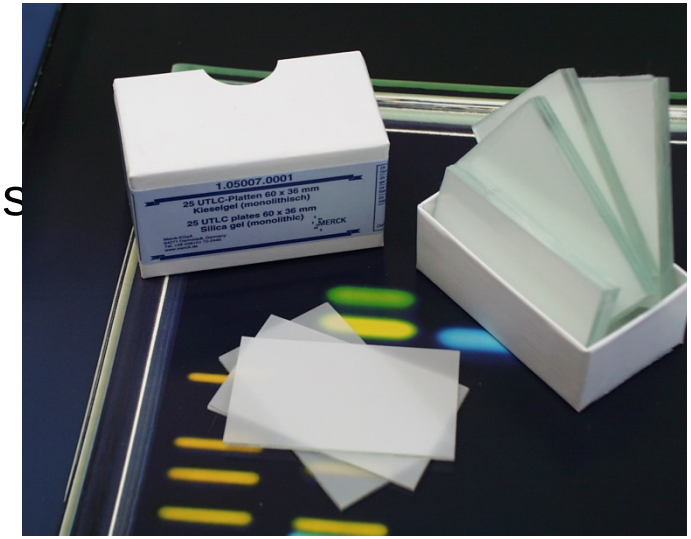
- Fine particle layers optimized for fast and efficient separations
- Wide range of chemically bonded phases
- Instrumentation for optimum sample application, development and detection
- Accurate and precise in situ quantification of chromatograms

Unique Product

Ultra-thin monolithic silica plate (UTLC)

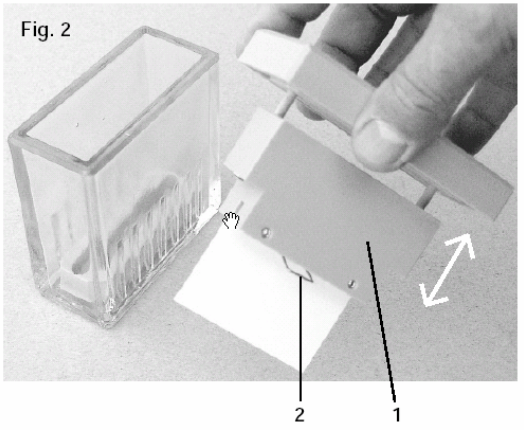
Features

- Ultra fast
- Very low sample volumes for precious samples
- Extremely sensitivity analysis in the **nl** range
- Binder free and stable in pure water



Applications

- Small simplier samples with low analyte concentration
- Drug discovery

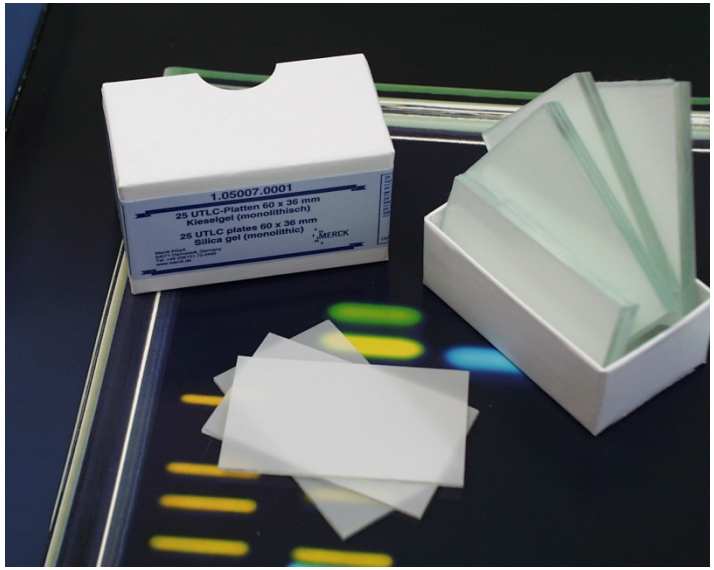


UTLC
Part of Monolithic Product Family

Chromolith CapRod®

Cromolith®

UTLC plates

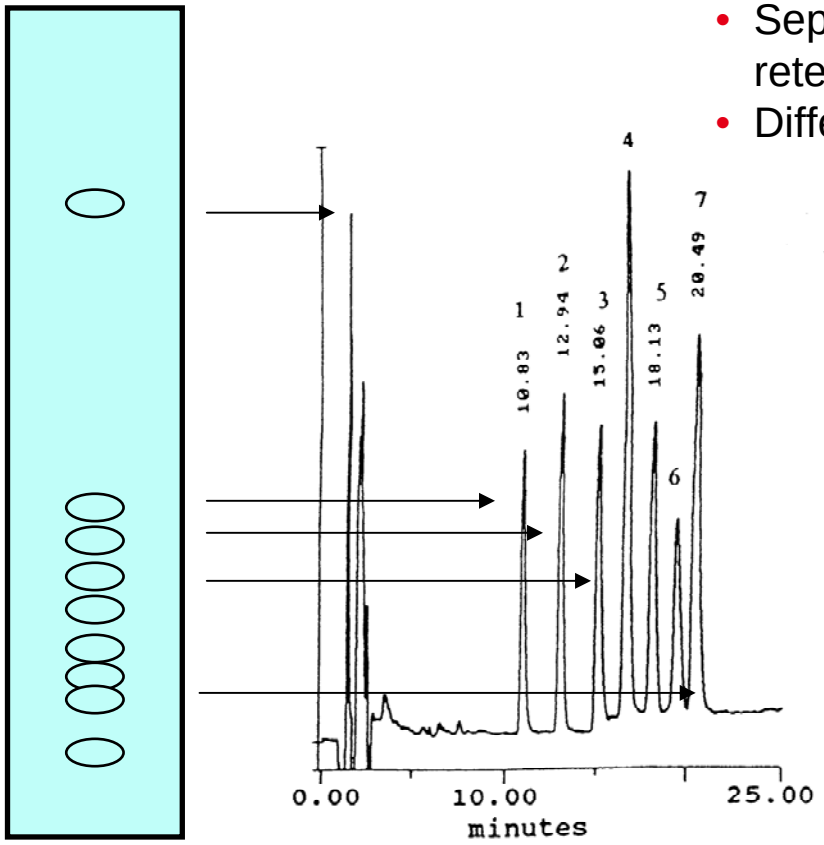


Cromolith®PrepRod

Fig. 1: Ready-to-use Chromolith® Prep Si column

Method tranfer from TLC to HPLC

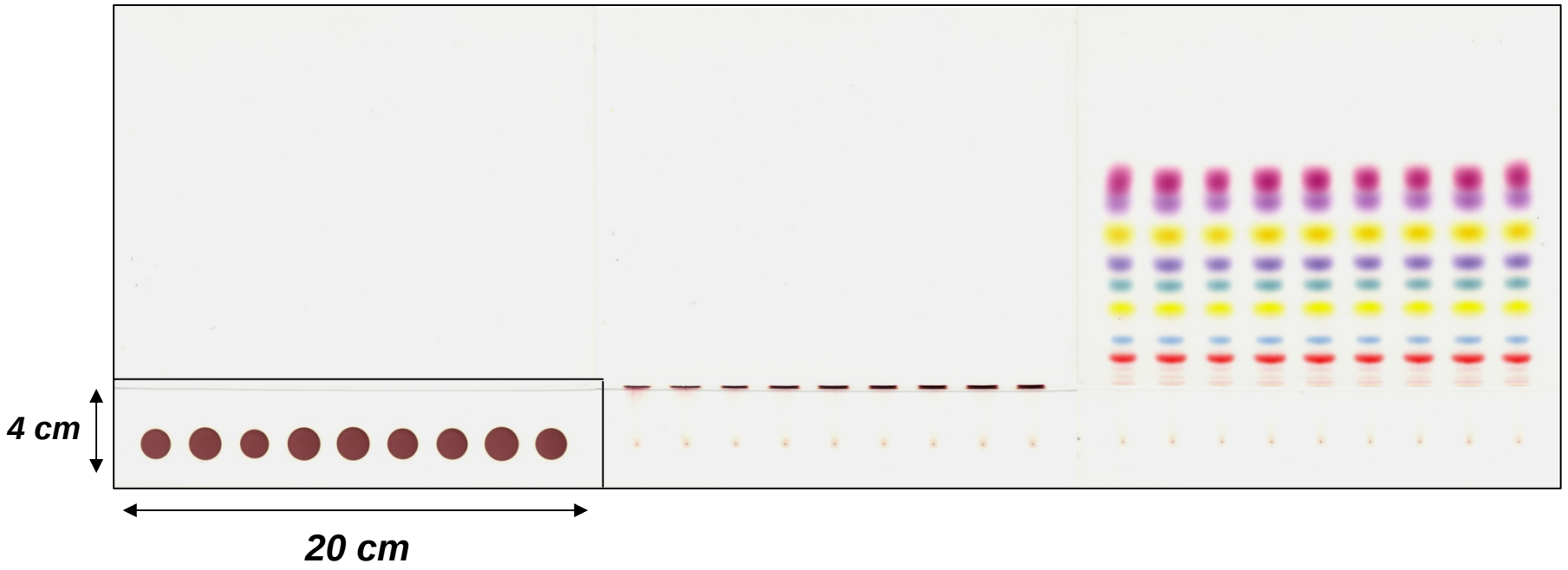
Fig. 1: TLC separation (left) and the corresponding HPLC separation (right)



HPLC and TLC

- Separations occur by the same retention mechanism
- Differences arise from:
  - Kinetic performance
  - Stationary phase format
  - Development mode vs. elution
    Disposable stationary phase (TLC)
  - Detection in the presence of the stationary phase (TLC)

Special Product
Concentrating Zone Plates

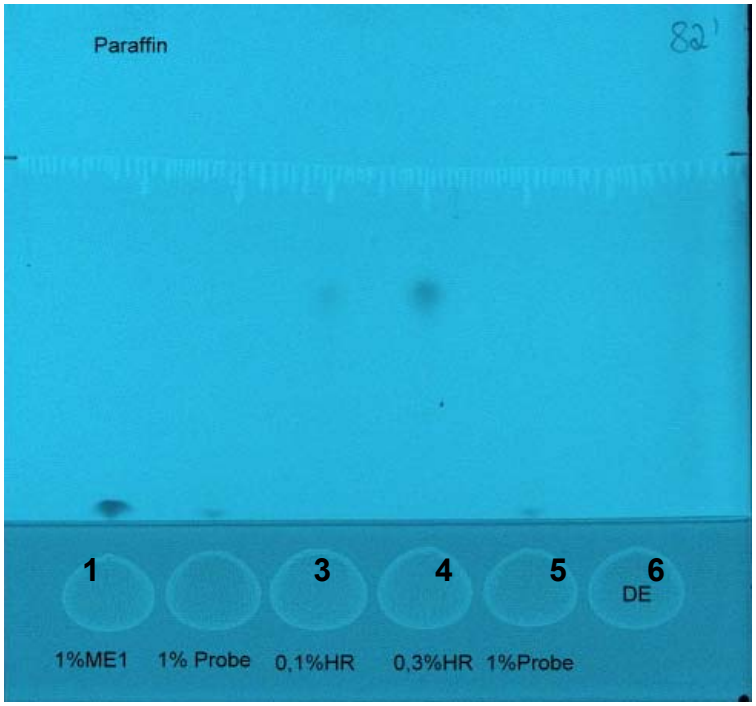


Application - Cosmetics
Stability testing of cosmetic ingredients

HPTLC for analysing in difficult matrices such as oils or fat

Is the ingredient X stable as paraffin formulation?

Solvent front



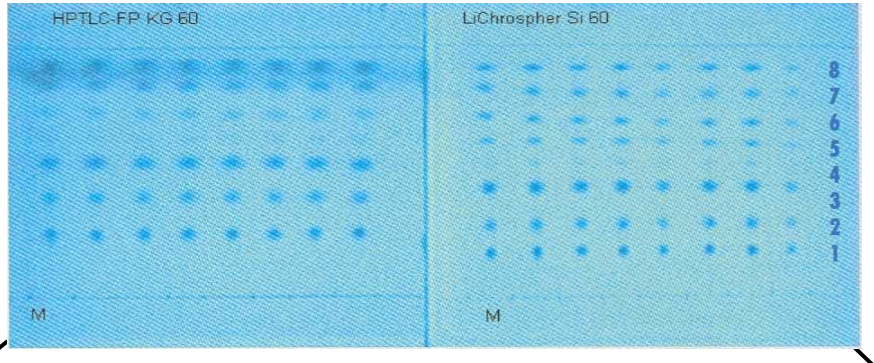
Sample: Ingredient (ester, di-ester)
Formulation: Paraffin
Solvent: Dichlormethan
Application: Linomat V (CAMAG)
Plate: HPTLC Silica gel 60 RP18 F254s Conz.
Mobile phase: Ethanol/Wasser 80:20
Drying time: 60 min
Migration distance: 5,0 cm
Migration time: 82 min
Samples: 2 µl (in Dichlormethan)

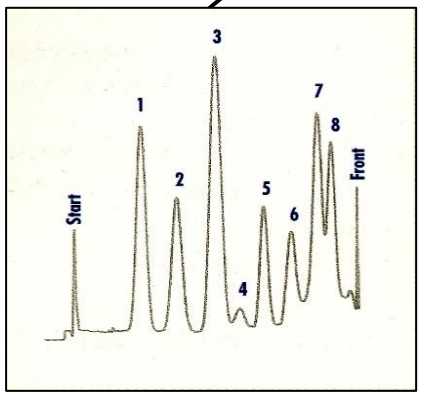
1 Pure ingredient (ME1) in Paraffin oil 1% (positive controle)
2 Sample in paraffin foil 01:01
3 HR in paraffin oil 0,10% (expected degradation product)
4 HR in paraffin oil 0,30% (expected degradation product)
5 Sample in paraffin oil 01:01
6 Pure ingredient (DE) in paraffin oil 1,00% (positive control but not visible under UV)

Data kindly provided by Merck Pigments & Cosmetics

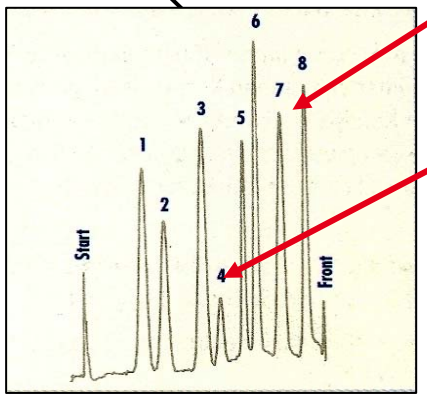
HPTLC LiChrospher® vs. LiChrosorb Highly Compact Bands

Comparison of a mixture of pharma substances





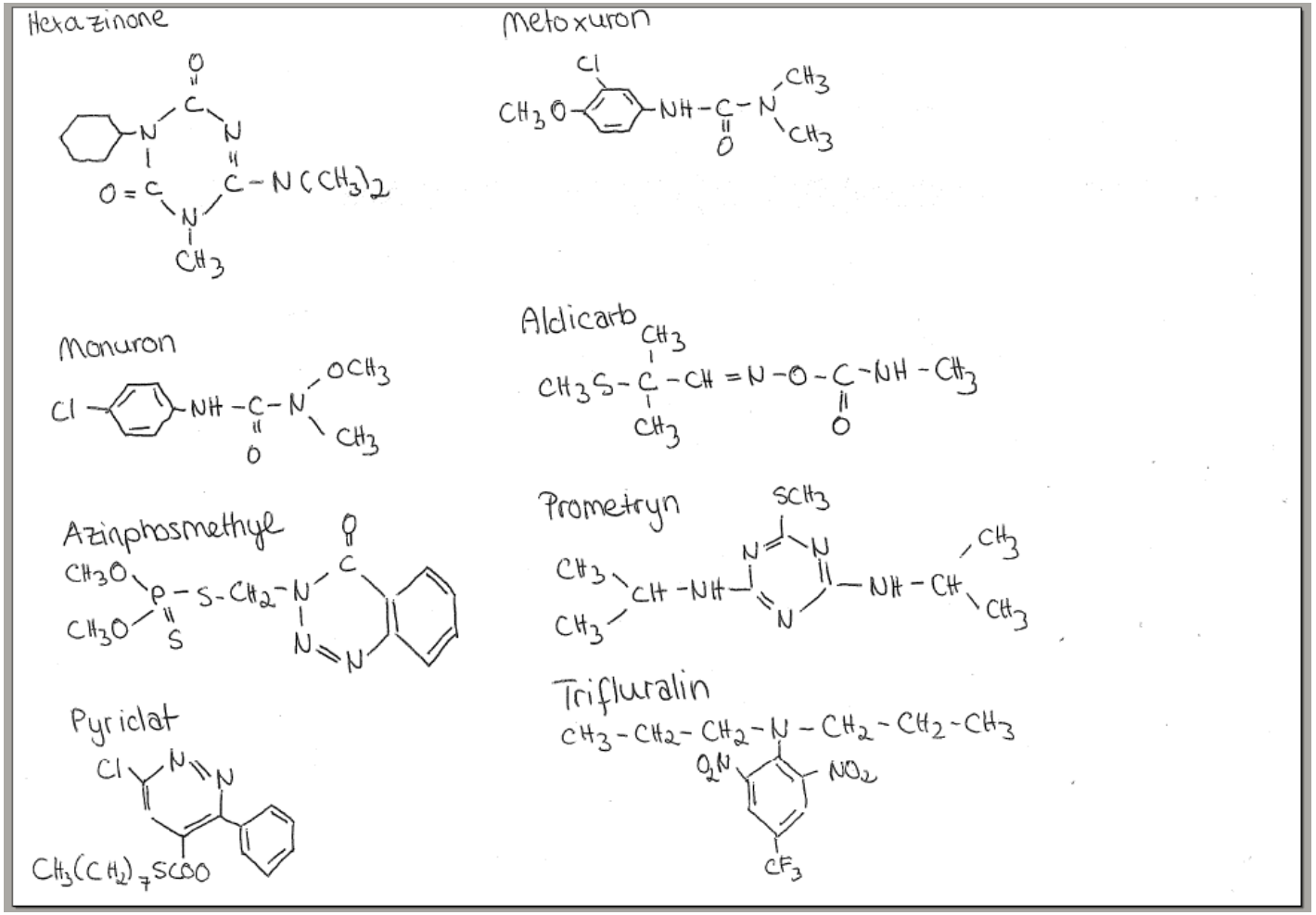
1 Hexazinone
2 Metoxuron
3 Monuron
4 Aldicarb
5 Azinphosmehtyl
6 Prometryn
7 Pyridat
8Trifluralin



HPTLC silica gel 60

HPTLC LiChrospher® silica gel 60

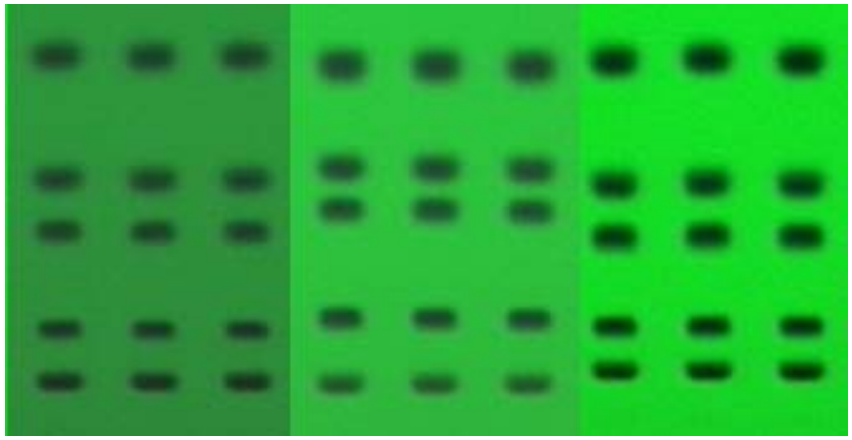
HPTLC LiChrospher® vs. LiChrosorb Highly Compact Band



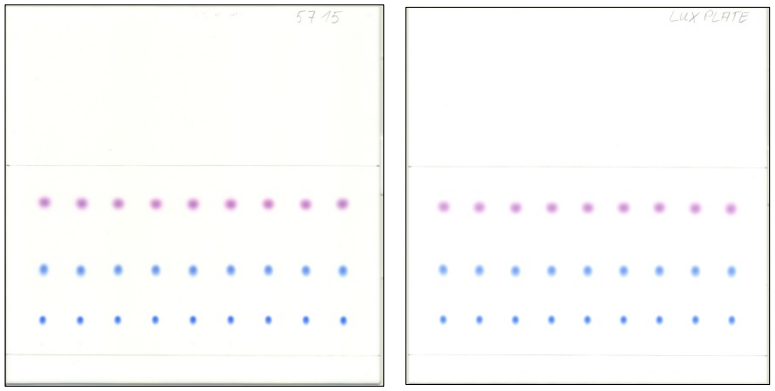
Special Product - LuxPlate®

- Higher content of fluorescent indicator for better contrast against background
- Highly robust, due to higher content of binder
- Comparable retention behaviour

Cassical silica 60 F_{254} | Competitor | LuxPlate®



LuxPlate® | Classical silica 60 F_{254}



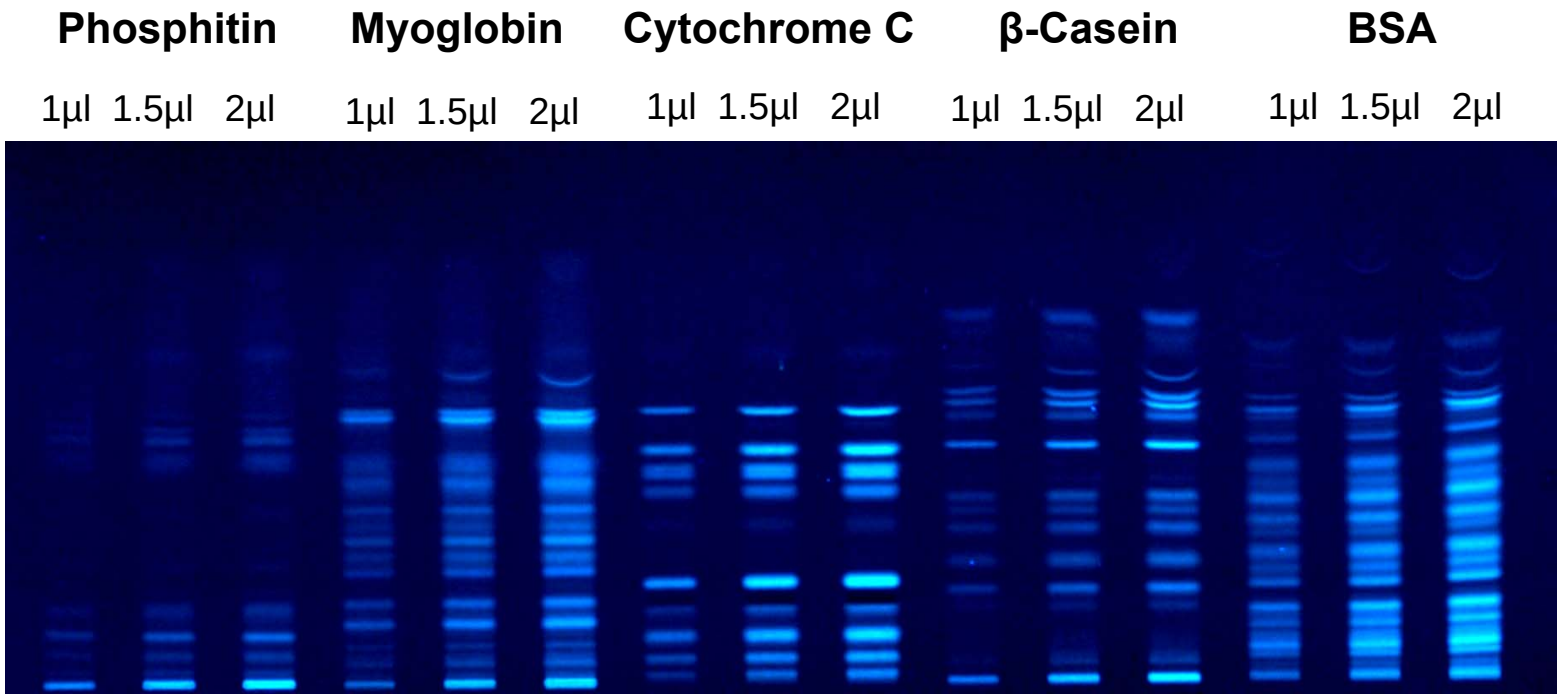
New Products

HPTLC Plates for Peptide Analysis

ProteoChrom®	Sorbent	Format	Layer	Backing	Special
1.05650 **HPTLC Silica gel F_{254s}**	High Performance Silica gel	20 x 10	100 µm	glass	Special binder
1.05651 **HPTLC Cellulose**	High performance Cellulose	10 x 10	100 µm	aluminium	High density layer

Why plates for analysis of protein digests & peptides?

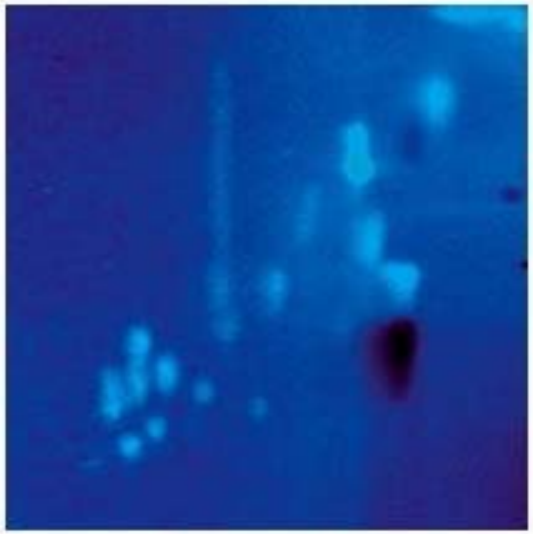
ProteoChrom®
Features



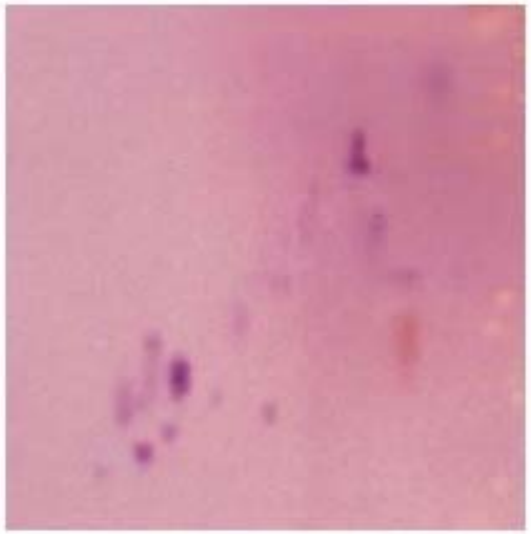
- Extra thin, extra smooth
- Robust, highly stable in water
- Include easy to follow, optimized protocols

ProteoChrom® HPTLC Cellulose
2 D separation of peptides

Fluorescamin



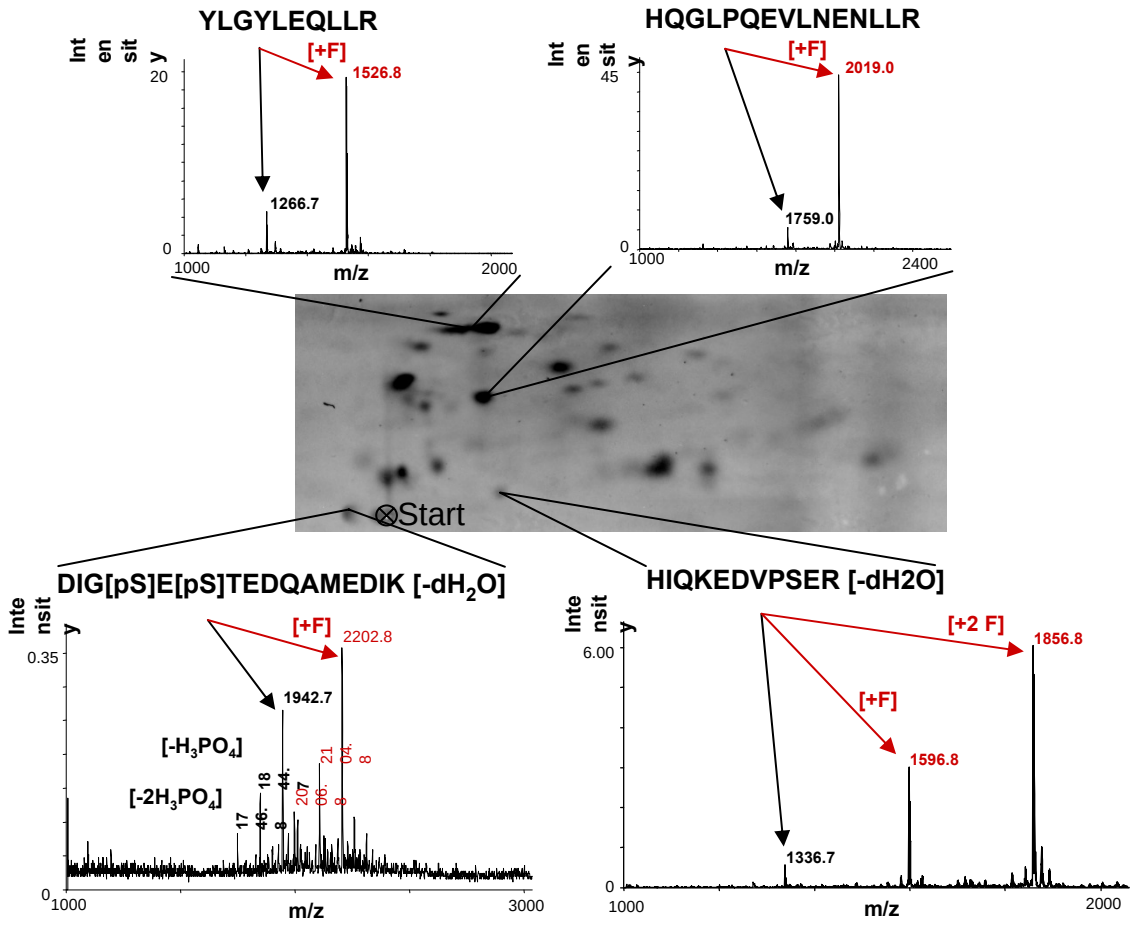
Ninhydrin



Sample volume: 5 µl
Concentration: 2 mg/ml
Application: Linomat V (CAMAG)
Migration distance: 5 cm
Migration time: 1st D: 45 min
2nd D: 50 min

- Fast, just 4 h from protein digest to result
- Validated for peptide separation

Mass Spectrometry directly from the Plate



FDA Applications with modern TLC

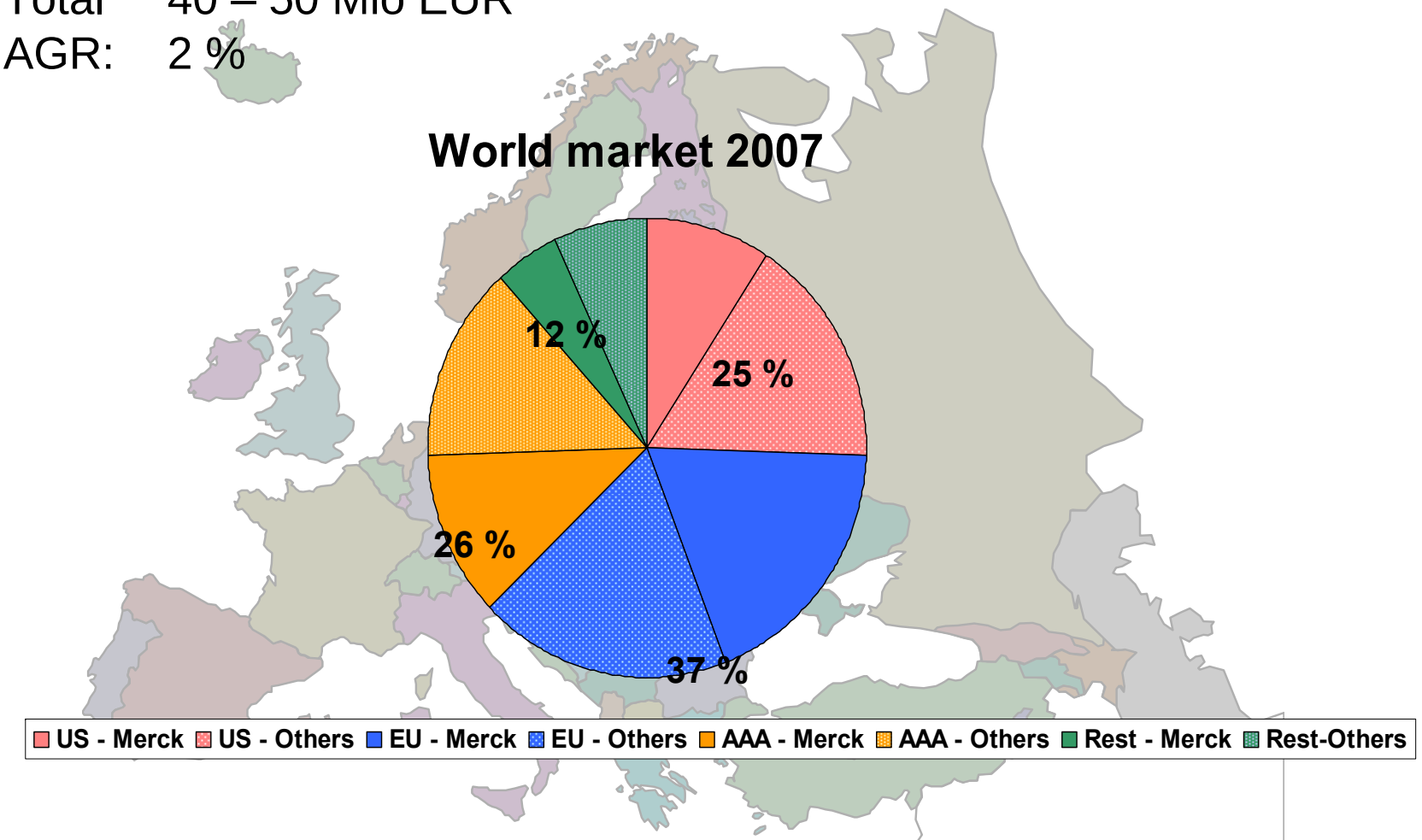
- Impurity and stability applications for synthetic drugs
- Fingerprinting of plant extracts
- Mycotoxins in foods
- Natural and synthetic food colors
- Vitamins

TLC - Challenge

Merck is market leader in a mature market

Market Thin Layer Chrom.

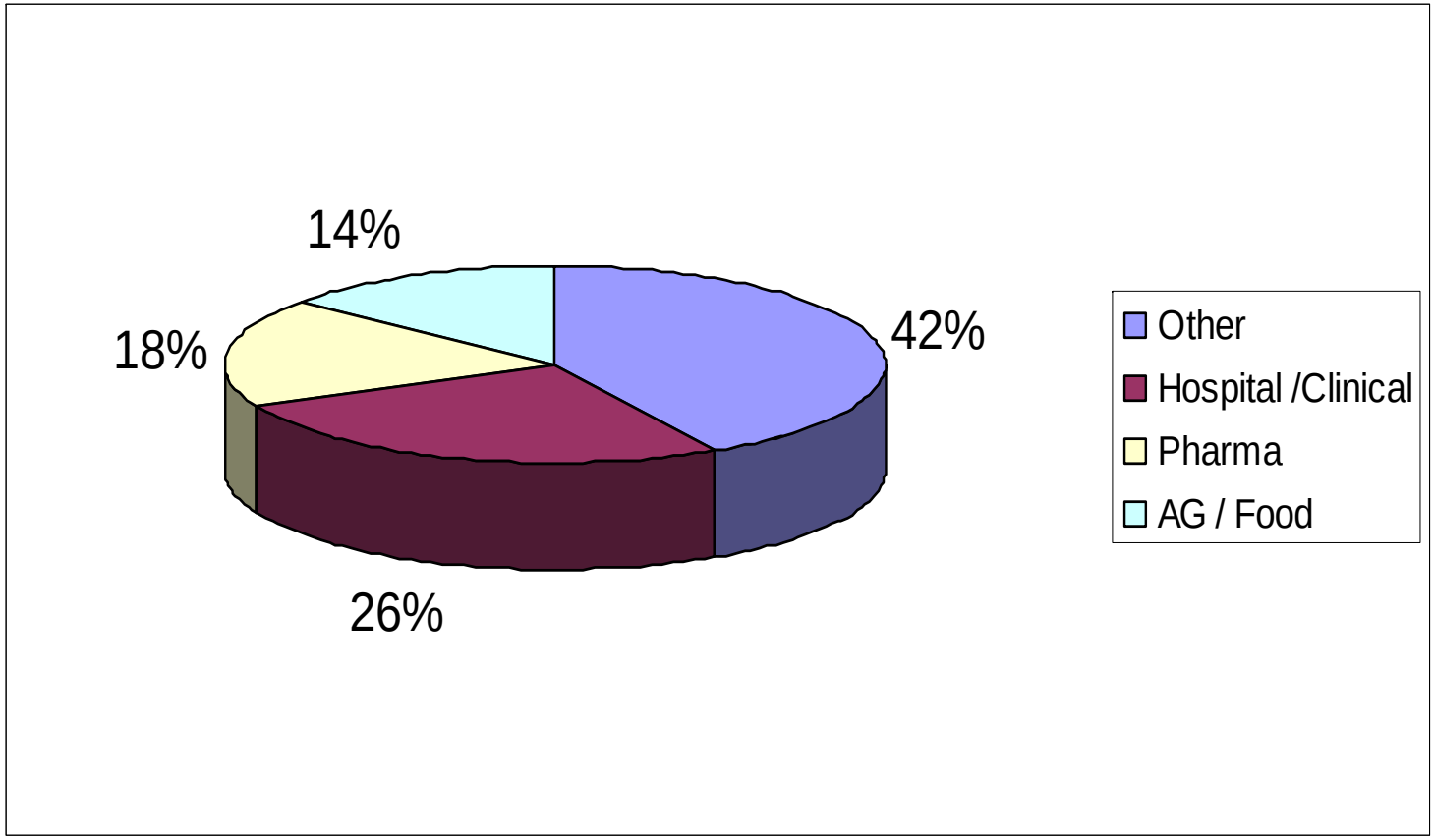
Total 40 – 50 Mio EUR
AGR: 2 %



We are by far the market leader in Thin layer chromatography!

Market Thin Layer Chromatography

Total 40 – 50 Mio EUR
AGR: 2 %



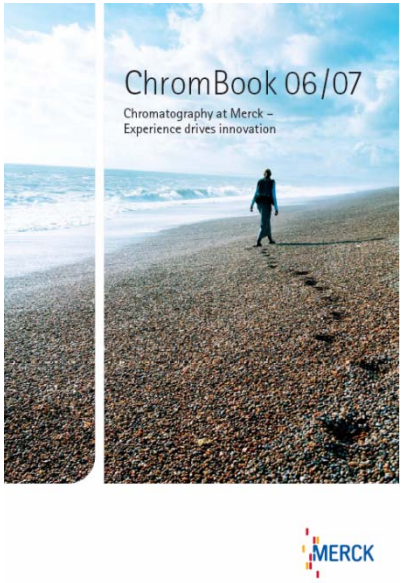
SDi Global Assessment Report 9th Edition, LCGC Oct.08

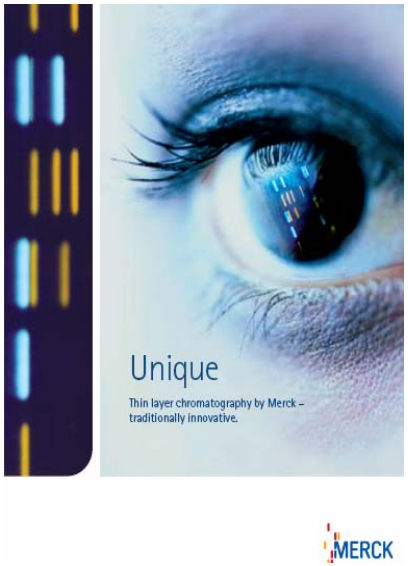
Summary

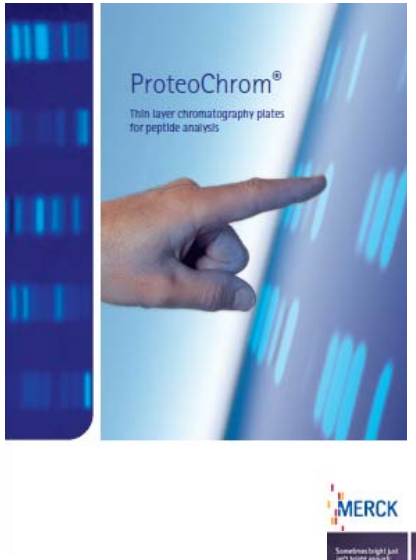
- Single use of stationary phase (TLC and HPTLC) minimizes sample preparation
- Parallel separations enhances sample throughput
- Ease of postchromatographic derivatization
- Can perform several screenings simultaneously for different analytes
- Direct use of biological detection possible
- Fast and low cost screening TLC- procedure used to identify samples that should be investigated further
- We use same raw material for TLC, HPLC and Prep HPLC, which makes easy to transfer method from TLC to HPLC

More on Thin-Layer Chromatography?

Chrombook 06/07







www.chromatography.merck.de